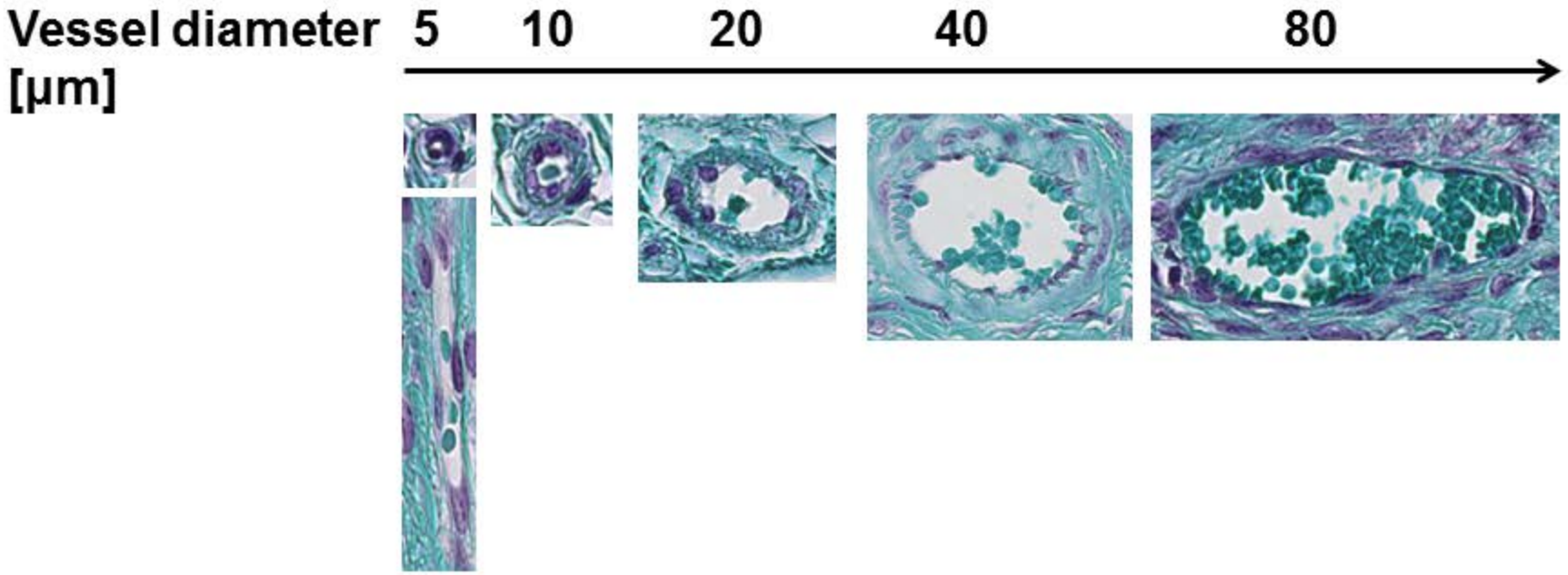
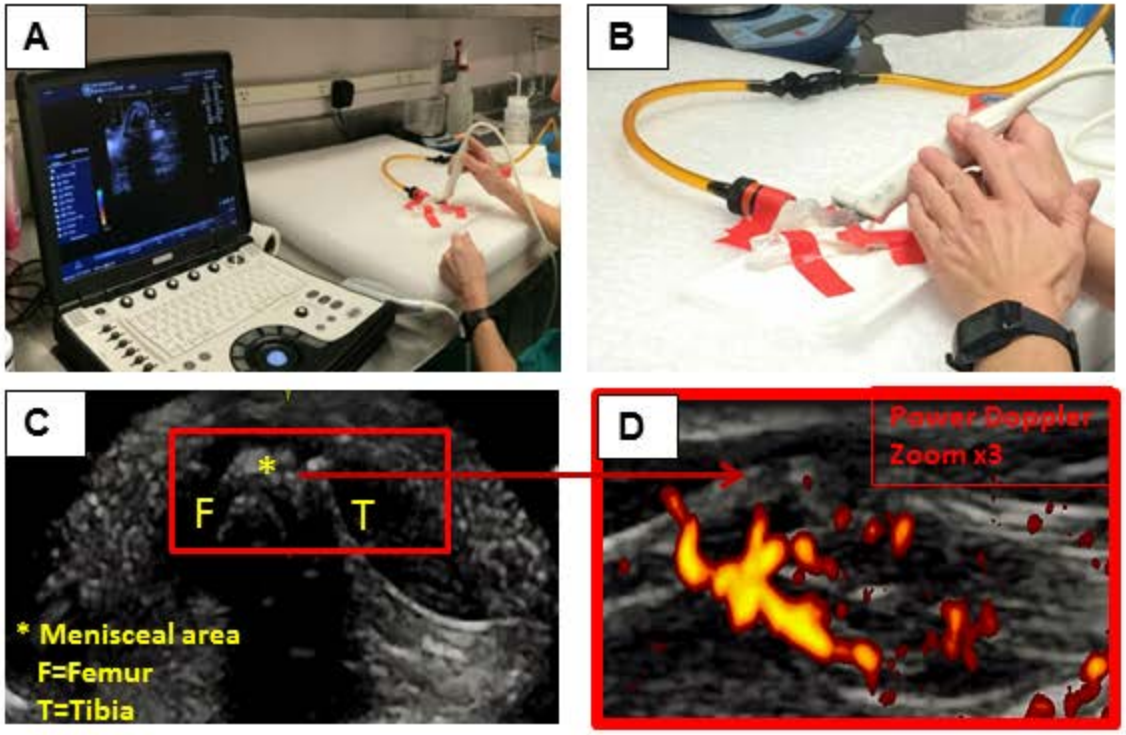
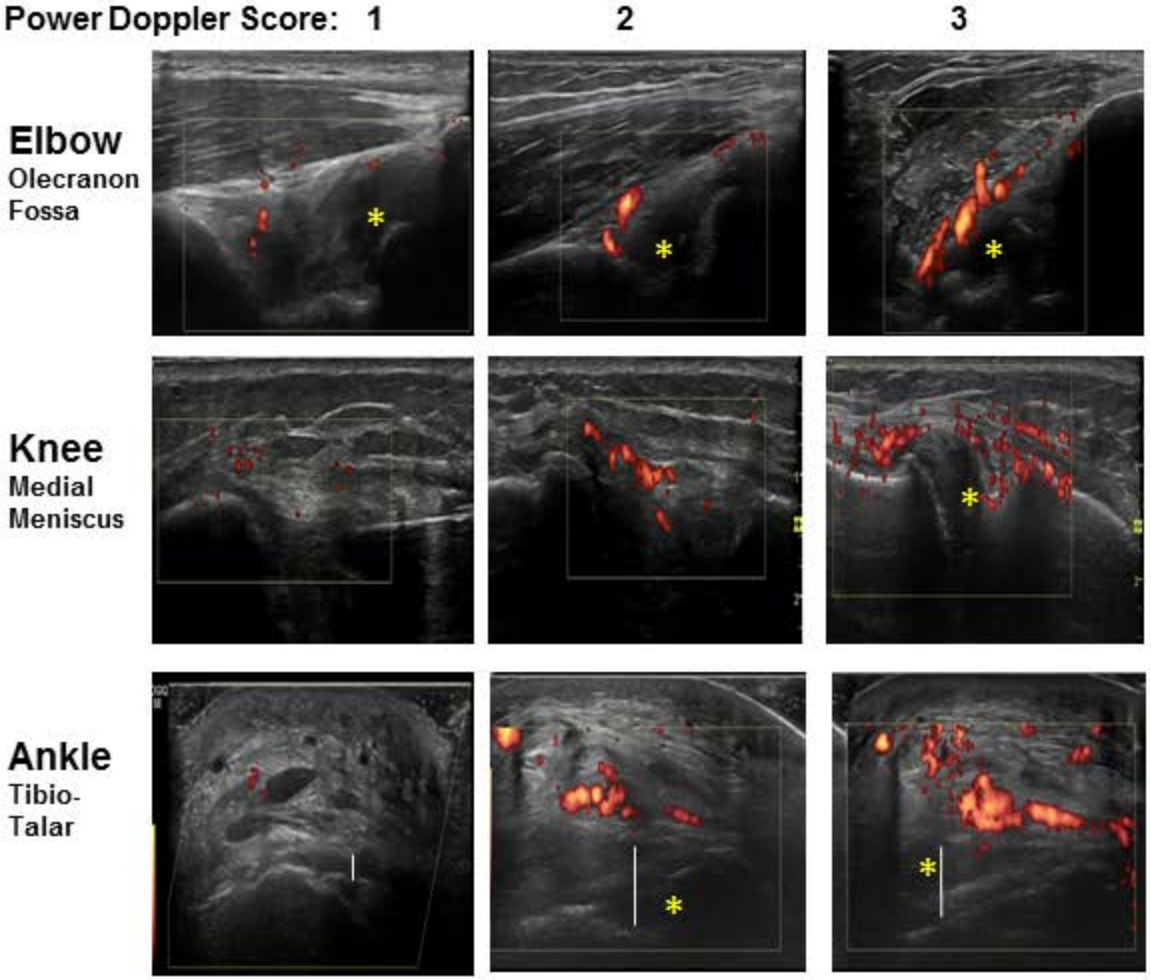


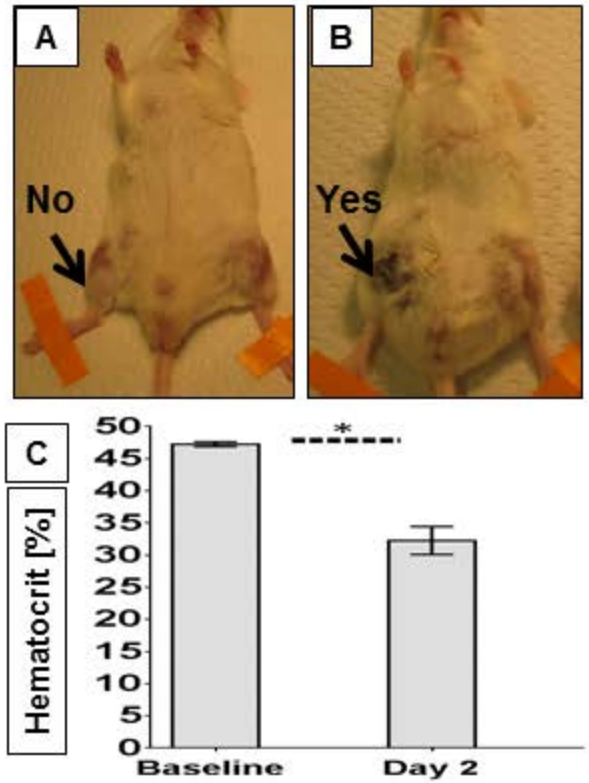
Supplemental Figure 1. Vessel morphology and methodology for vessel size determination. The right knees of FVIII-deficient mice were subjected to subpatellar puncture. Vessel characteristics were studied histologically after Safranin-0-Green staining at 40-fold magnification. Individual vessel diameters were determined by ruler. For elliptical vessels, the larger diameter was recorded unless it exceeded the smaller diameter by > 2.5-fold. In such cases the smaller diameter was recorded (see the 2 examples for 5 µm scoring)



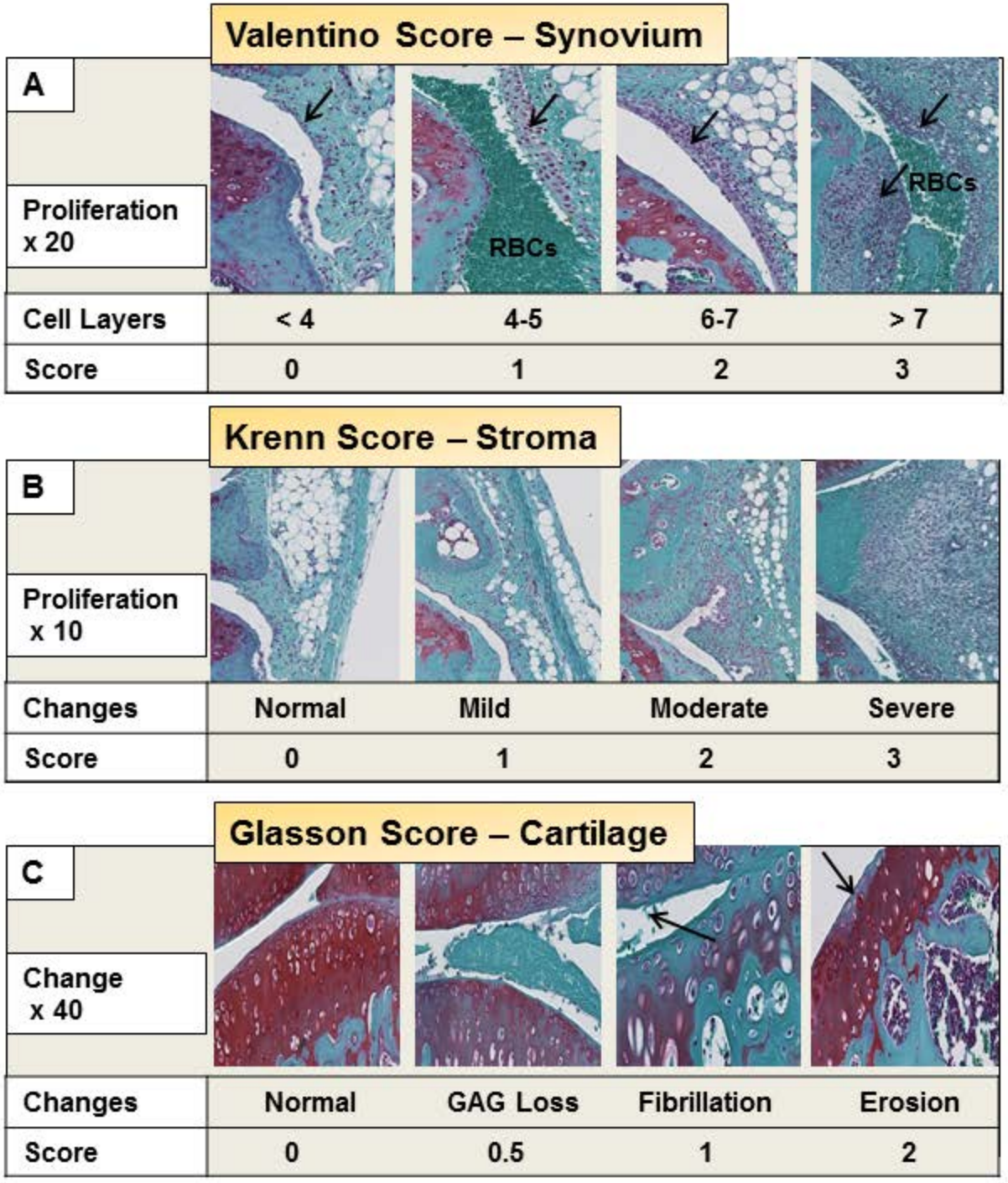
Supplemental Figure 2. Vascular flow detection in the knee joint of FVIII-deficient mice by high resolution ultrasound and power Doppler (PD). A) Positioning of the anesthetized mouse and visualization of the knee joint after hair removal using ultrasound gel with the hockey stick transducer probe at 16 MHz. B) Alignment of probe with tibia and medial menisceal area longitudinally (long axis) at ~50% knee flexion. C) Magnification of the visualized area with the zoom feature. D) PD assessment of vascularity within the magnified area. E) Use of power pulse waves in sentinel vessels to assure vascular flow and rule out artefact.



Supplemental Figure 3. Soft tissue perfusion was assessed by power Doppler signal in 3 different anatomical locations in each hemophilia joint, and scored semi-quantitatively (0= no signal; 1= small spots; 2= confluent vessels in <50% tissue of interest; 3= confluent vessels in ≥ 50% tissue of interest). Location signals in each joint were added to a total joint score (min 0; max 9). Depicted are scoring examples in specific joint locations. (*) indicates effusion; white line indicates widened tibio-talar ankle joint space.



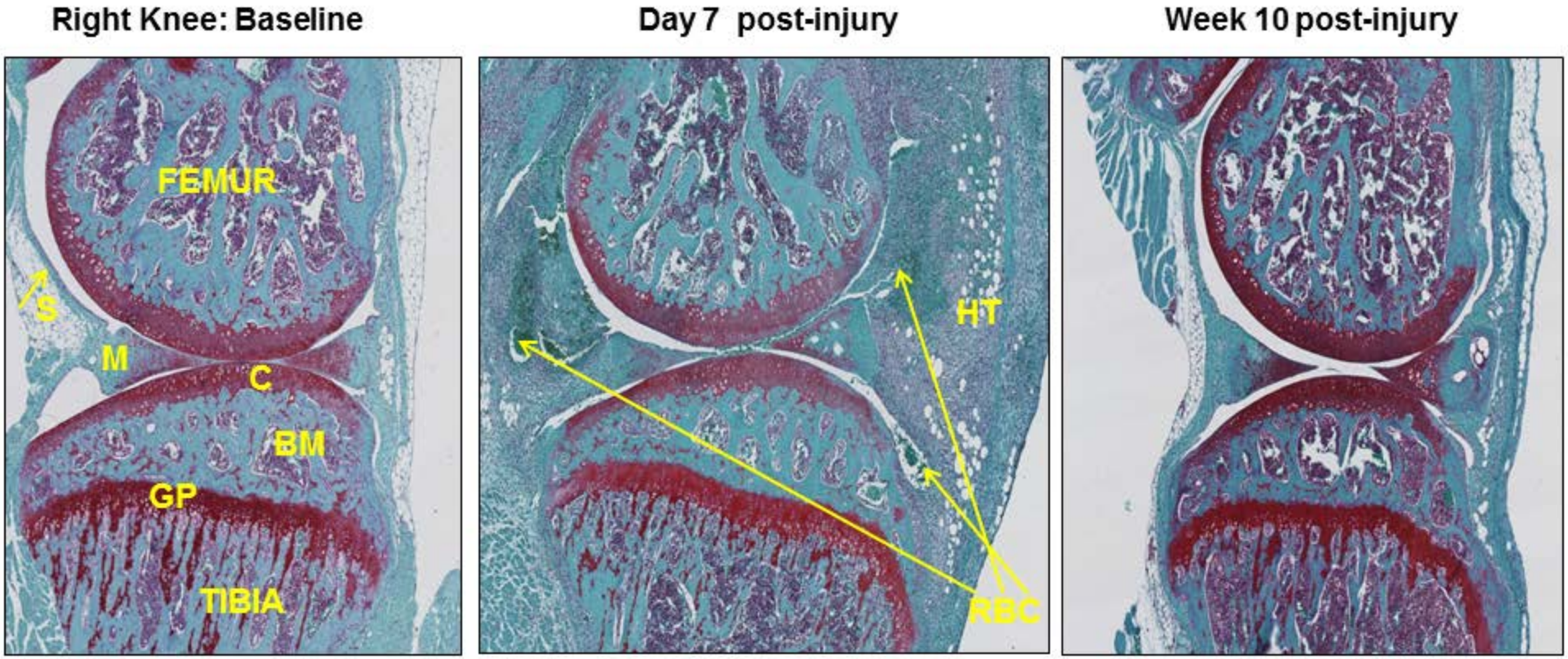
Supplemental Figure 4. Blood loss after knee puncture in FVIII-deficient mice. The right knees of FVIII-deficient mice were subjected to subpatellar puncture. Visual joint inspection for presence of bleeding A) before and B) 2 days after puncture. C) Decrease of hematocrit from baseline 2 days post knee puncture as a measure of intra-and peri-articular blood loss (≥25 mice per group). Statistical significance was performed with the Mann Whitney test. Error bars represent standard error of the mean. (*) $p < 0.0001$



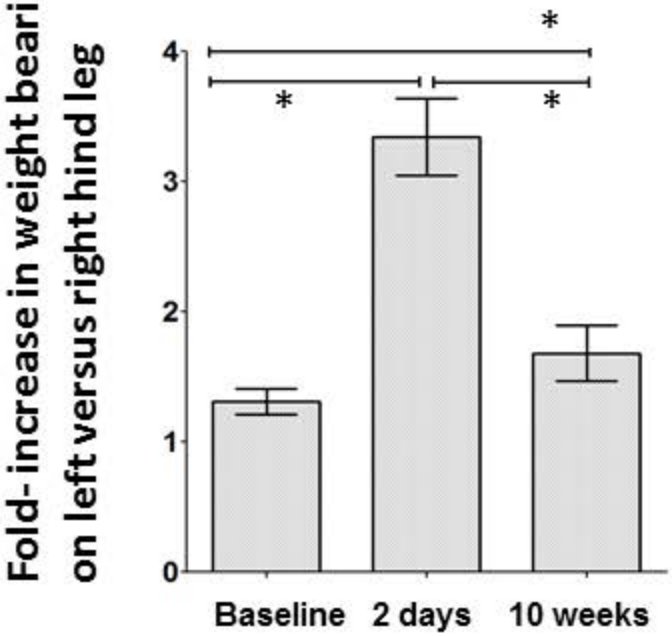
Supplemental Figure 5. Scoring algorithms for synovial, stromal and cartilage changes. The right knees of FVIII-deficient mice were subjected to subpatellar puncture. Representative examples after bleed induction of increasing grades of histological: A) synovial, B) stromal and C) cartilage changes are shown following Safranin-0-Green staining.

Semi-quantitative scoring was administered according to A) Valentino-, B) Krenn- and C) Glasson for synovium, stroma and cartilage, respectively.

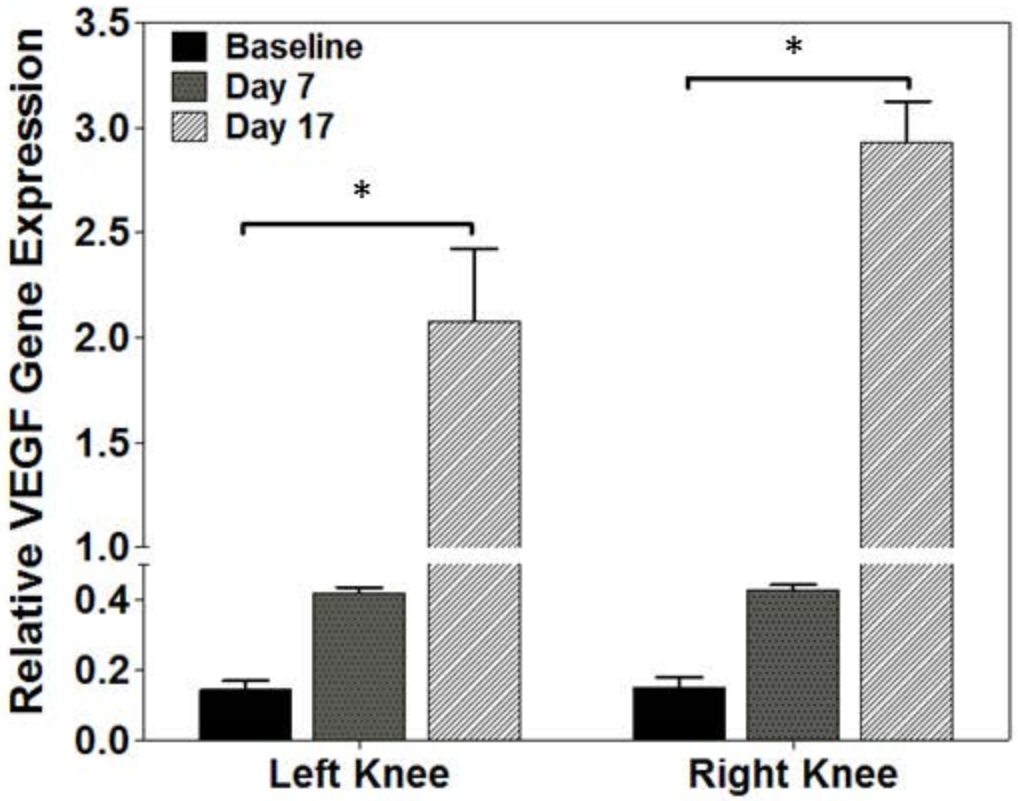
The scoring algorithm for each system is shown with representative examples from the study. For synovial and stromal changes, grade 3 represents the most severe changes in the Valentino- and Krenn-scoring systems while, for cartilage, grade 6 is worst according to Glasson-scoring. For cartilage, only grading examples up to a score of 2 are shown since this was the highest score identified in our experimental series (erosions). Arrows (→) represent pertinent changes. Glycosaminoglycan: GAG. Red Blood Cells: RBCs.



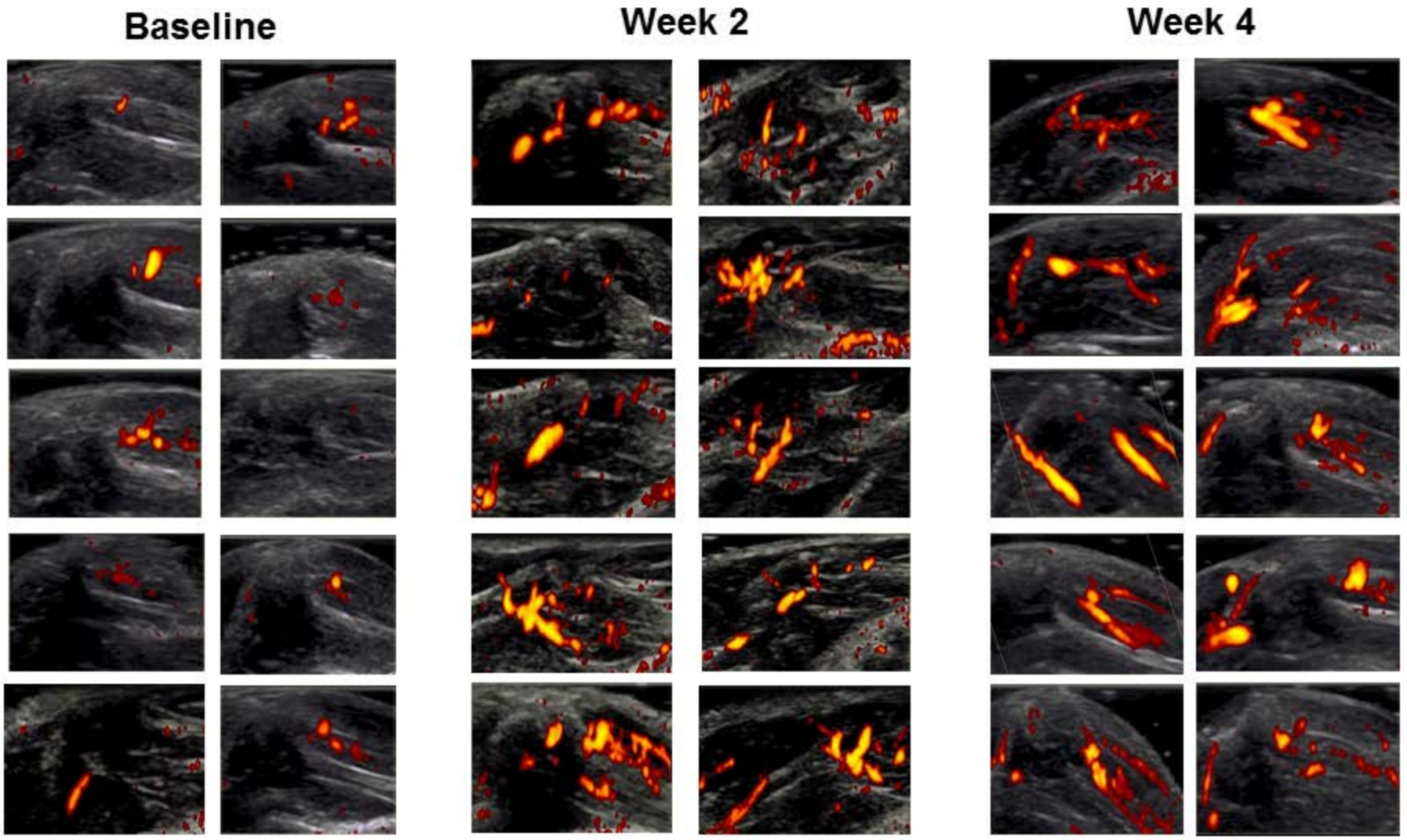
Supplemental Figure 6. Time course of soft tissue changes. Representative examples of the injured right knee in FVIII-deficient mice at baseline, 7 days and 10 weeks after needle puncture. Findings of hypercellularity and tissue changes observed on day 7 return to near baseline 10 weeks post-injury. Safranin-0-Green staining at 4-fold magnification. BC, Blood Cells; BM, Bone Marrow; C, Cartilage; GP, Growth Plate; HT, Hypercellular Tissue; M, Meniscus; S, Synovium. (→) pointing to synovial lining (normal 1-3 cell layers).



Supplemental Figure 7. Changes in weight bearing of the injured right hind leg following induced hemarthrosis in FVIII-deficient mice. The right knees of FVIII-deficient mice were subjected to subpatellar puncture. Weight bearing capacity of the injured right hind leg was assessed at baseline, 2 days and 10 weeks post injury (n=5-7 mice per group). Unilateral weight bearing differences (gm) were recorded and averaged over at least 40 intervals (15 sec for each interval). Values were expressed as ratio of left/right leg weight bearing capacity. Statistical significance was performed with the Student T-test. * denotes stastical significance ($p \leq 0.5$) Error bars represent the standard error of the mean.



Supplemental Figure 8. Gene expression of vascular endothelial growth factor (VEGF) in synovial tissue of FVIII-deficient mice after induced hemarthrosis. Reverse transcription polymerase chain reaction of mRNA for VEGF in synovial tissue isolated from the injured right knee and the control left knee at baseline, and after 7 and 17 days. Values are expressed as mean relative increase of gene expression over glyceraldehyde-3-phosphate dehydrogenase. Statistical significance was determined by Mann Whitney test. * denotes statistical significance. Error bars represent standard error of the mean; n = 4 repetitions (synovial tissue from 5-7 mice per time point).



Supplemental Figure 9. Vascular architecture and joint tissue perfusion. Changes following induced hemarthrosis in FVIII-deficient mice. The right knees of FVIII-deficient mice were subjected to subpatellar puncture. Vascularity and perfusion patterns were studied using high resolution musculoskeletal ultrasound and Power Doppler. Ultrasound images with Power Doppler visualizing vascular flow of knees at baseline, and 2 and 4 weeks after injury are shown (n= 10 per group).